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REVIEW

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Oviposition strategies of conifer seed chalcids in relation to host phenology

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Abstract Insects are considered the most important predators of seed cones, the female reproductive structures of conifers, prior to seed dispersal. Slightly more than 100 genera of insects are known to parasitize conifer seed cones. The most diverse (i.e., number of species) of these genera is *Megastigmus* (Hymenoptera: Torymidae), which comprises many important seed pests of native and exotic conifers. Seed chalcids, *Megastigmus* spp., lay eggs inside the developing ovules of host conifers and, until recently, oviposition was believed to occur only in fertilized ovules. Ovule development begins just after pollination, but stops if cells are not fertilized. The morphological stage of cone development at the time of oviposition by seed chalcids has been established for many species; however, knowledge of ovule development at that time has been documented for only one species, *M. spermotrophus*. *Megastigmus spermotrophus* oviposits in Douglas-fir ovules after pollination but before fertilization. Unlike the unfertilized ovules, those containing a *M. spermotrophus* larva continue to develop, whether fertilized or not, stressing the need to broaden our understanding of the insect–plant interactions for this entire genus. To achieve this task, we reviewed the scientific literature and assembled information pertaining to the timing of oviposition and to the pollination and fertilization periods of their respective host(s). More specifically, we were searching for circumstantial evidence that other species of *Megastigmus* associated with conifers could behave (i.e., oviposit before ovule fertilization) and impact on female gametophyte (i.e., prevent abortion) like *M. spermotrophus*. The evidence from our compilation suggests that seed chalcids infesting Pinaceae may also oviposit before ovule fertilization, just like *M. spermotrophus*, whereas those infesting Cupressaceae seemingly oviposit after ovule fertilization. Based on this evidence, we hypothesize that all species of *Megastigmus* associated with Pinaceae can oviposit in unfertilized ovules, whereas those exploiting Cupressaceae cannot, and thus oviposit only in already fully developed fertilized seeds. Furthermore, we predict that the presence of a larva in unfertilized ovules of all Pinaceae will influence the development of the female gametophyte by preventing its abortion. This influence on the Pinaceae can be interpreted as an ability to parasitize any of the potential seeds present in a seed cone, and as such represents a much more efficient oviposition strategy than searching and locating only fertilized seeds. Concomitantly, this ability has likely led to an overestimation of the impact of the species of seed chalcid infesting Pinaceae on seed production.

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Introduction

The fitness of insects parasitizing discrete resources such as seed cones or seeds can, on occasion, be linked to the synchrony between the timing of oviposition and host

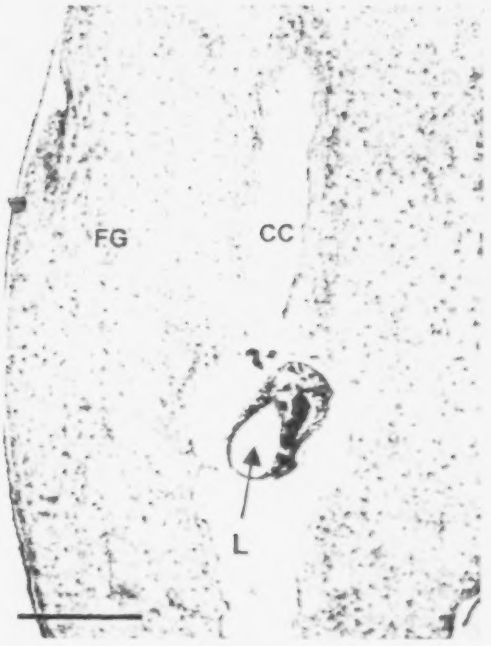


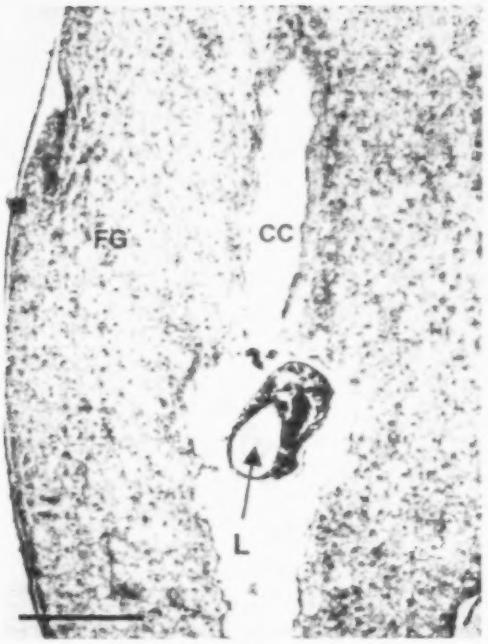
Fig. 1 Micrograph section of a *Megastigmus spermotrophus* larva developing in the female gametophyte of a pollinated seed of *Pseudotsuga menziesii*. *L* larva, *FG* female gametophyte, *CC* corrosion cavity. Stained with Safranin-Fast Green. *Scale bar* = 1 mm (G. Rouault)

Fig. 2 Radiograph of Douglas-fir seeds infested by *Megastigmus spermotrophus* larvae, pupae and adults about to emerge. *ES* empty seeds, *FS* filled seeds, *L* larva, *N* pupae, *F* female, *M* male; *scale bar* = 4 mm (G. Rouault)

Fig. 3 Female of *Megastigmus schimitschekt* Novitzki ovipositing in a cone of *Cedrus*. *Scale bar* = 1 mm (S. Boivin)

development (McClure et al. 1998). The genus *Megastigmus* Dalman (Hymenoptera: Torymidae) currently comprises 126 species; about one-third of these species are seed-feeders of conifers (Grissell 1999; Roques and Skrzypczynska 2003). These seed feeders are endoconophytic species (Turgeon et al. 1994) and, as such, all immature stages develop inside the seed (see Figs. 1 and 2). The timing of oviposition in relation to cone external morphology has been established for many species of seed chalcid (Hedlin 1956; Keen 1958; Roques 1983; Roques et al. 1984), yet little is known about the internal development of the seed, which is where eggs are deposited and larval and pupal development occurs. The host range of each species is typically limited to one genus, although on rare occasions it consists of two genera (Grissell 1999; Roques and Skrzypczynska 2003). For example, some invasive species have colonized native conifers in the area of introduction that belong to a genus with a phenological development comparable to that of the host tree in the area of origin (Fabre et al. 2004). Seed chalcids have developed strategies to survive years of low cone production or low pollination success. These include prolonged diapause and parthenogenesis (Hussey 1955; Roques 1981, 1989; Annila 1982).

On conifers, females lay eggs directly inside ovules by inserting their ovipositor (Fig. 3) through the young cone scales (Milliron 1949; Hussey 1955; Keen 1958; Hedlin et al. 1980; Roques 1981). Although multiple infestations (i.e., more than one egg per ovule) appear common, there is never more than one mature larva per seed (Milliron 1949; Keen 1958). The only nutritive tissues available to all five instars are the female gametophyte and the seed embryo. Oviposition and larval development of all seed chalcids parasitizing conifers were assumed to occur ex-



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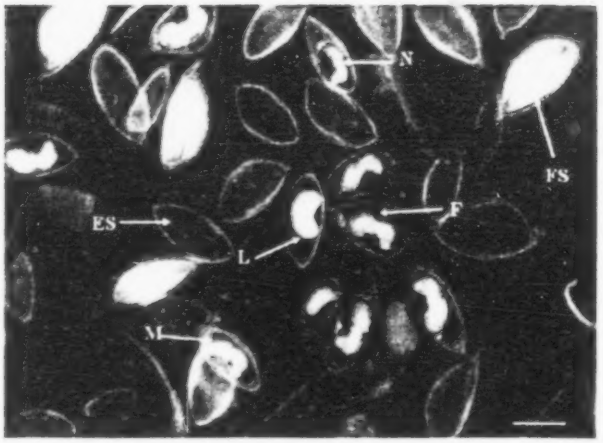

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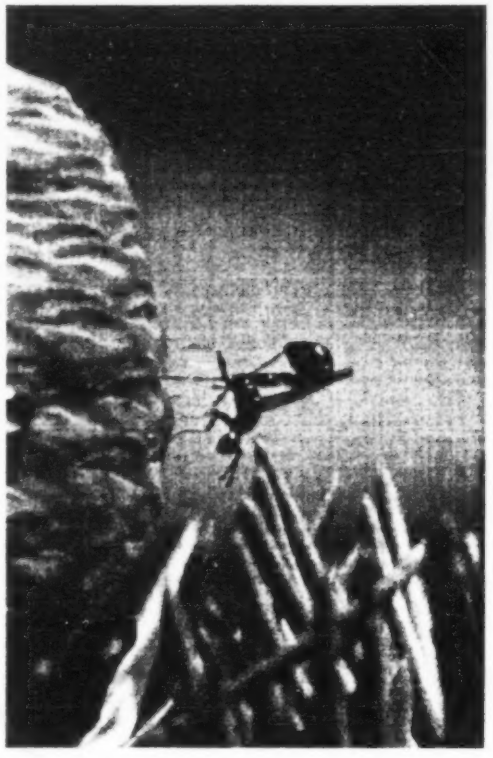

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clusively in fertilized ovules (i.e., containing a conifer embryo) (Hussey 1955), until Niwa and Overhulser (1992) and Rappaport et al. (1993) experimentally demonstrated that normal development of *Megastigmus spermotrophus* Wachtl, the Douglas-fir seed chalcid, was possible in unfertilized ovules (Fig. 4), which normally should have aborted. This raises the question: Do all other species of seed chalcids exploiting conifers also oviposit before fertilization and do they have the ability to develop in unfertilized ovules?

Megastigmus spermotrophus, a native of North America, is probably the most notorious seed chalcid because of its considerable impact on the production of *Pseudotsuga menziesii* (Mirb.) Franco seeds in European seed orchards, where it has been introduced accidentally (Rappaport and Roques 1991; Jarry et al. 1997). Eggs of *M. spermotrophus* are laid in the ovules within the developing gametophyte (Fig. 4b–*C*) in May. Larval development begins before the female gametophyte is fully developed (Fig. 4b–*D*) and, despite the presence of a larva, the female gametophyte continues to develop (Rouault 1998). Larval development ends about 2 months later, when the entire nutritive tissue has been consumed (Hussey 1955; Roques 1981). *Megastigmus spermotrophus* overwinters in diapause as a fifth instar. Larvae may extend the winter diapause for one to four more years in seeds released on the ground, enabling populations to survive in years with either no or low cone production (Annila 1982; Roques 1989, 1990; Roux 1995). For chalcids ending diapause, pupation occurs in mid-spring. Adult emergence occurs in late spring or early summer (Hussey 1955; Roques 1981). Most other seed chalcids exploiting Pinaceae also emerge from seeds fallen to the ground, however on Cupressaceae, emergence of *Megastigmus* spp. occurs before seed release, and larvae can remain in diapause in seeds within unopened serotinous cones still hanging on the tree (Battisti et al. 2003). Except for *M. spermotrophus* and Douglas-fir, the relationships between ovule development and seed chalcids are poorly understood.

Conifer reproductive cycles (from floral initiation to seed release) range from 2 to 4 years (Singh 1978; Owens and Blake 1985). In the ovule, initiation of the female gametophyte either occurs before pollination, coincides with pollination or, in some cases, is delayed for as much as a year (Singh 1978). After several weeks of free nuclear division, cell walls appear between the haploid nuclei, forming a multicellular female gametophyte (Fig. 4a–*B*). The mature gametophyte is composed of a haploid tissue in which archegonia develop (Singh, 1978; Owens and Blake 1985). These contain the egg cells (Fig. 4a–*D*), which once mature are penetrated by the pollen. Although most female gametophyte growth ends at the time of fertilization, histological and physiological changes continue. Following embryo formation (Fig. 4a–*P/E*), the female gametophyte tissue stores starch, lipid, and protein (Krasowski and Owens 1993; Anderson and Owens 2001; Chiwocha and von Aderkas 2002).

In many conifers with a 2-year cycle, pollen plays no role in female gametophyte development and ovules develop normally in unpollinated female cones. In the absence of pollen (Fig. 4a-UnR/D), female gametophytes grow to their usual final size; however, 2 weeks after fertilization would normally have taken place, they abort, leaving "empty" seeds (Owens and Blake 1985; Powell and Tosh 1991). In *Picea* spp., ovules develop partially before pollination and will eventually abort if not pollinated (Owens and Molder 1979; Owens and Blake 1984, 1985; Runions and Owens 1999a, 1999b). Species of the genera *Cupressus*, *Juniperus*, *Pinus*, and *Thuja* reportedly require the presence of pollen for a normal female gametophyte and ovule development and a certain proportion of pollinated ovules to avoid cone abortion (Ottley 1909; McWilliams 1959; Singh 1978; Owens et al. 1981, 1982, 1990; Owens and Blake 1985; Arista et al. 2001).

Our objective was to determine whether the relationship between oviposition of other species of *Megastigmus* exploiting conifer seeds and seed cone development resembled that of *M. spermotrophus* on Douglas-fir. Our approach consisted of reviewing the literature to find systems for which information about the periods of seed chalcid oviposition and those of ovule and female gametophyte development of the conifer host were sufficiently detailed to establish with some level of confidence the most likely host–insect phenological relationship. More specifically, we asked whether these other species of *Megastigmus* would also have the opportunity to oviposit before fertilization. We assumed that this opportunity was a strong indication of the ability to develop in unpollinated ovules. A total of 12 systems that encompassed all three primary types of reproductive cycles for conifers and included representative species from two (i.e., Pinaceae and Cupressaceae) of the seven families of conifers were examined. Based on these compilations, we formulated hypotheses and predictions on the significance of observed patterns on the oviposition strategy of seed chalcids and on the importance this has for the assessment of chalcid impact on seed production.

For each coniferous host, we established the following parameters: (1) duration of the reproductive cycle (defined as the period from pollination to seed release); (2) pollination period (which begins with pollen dispersal);(3) fertilization period (defined as the time a germinated pollen grain releases its gametes in an ovule); (4) female gametophyte development period; and (5) timing of seed release (Fig. 5a, b). For all the conifers included in this study, floral initiation occurs in the year prior to pollination. The reproductive cycle of each conifer was derived from studies carried out within its natural range.

For each chalcid species, we established the approximate time of chalcid oviposition on the selected conifers on the basis of biological information provided in the literature from studies or surveys that have been conducted in the natural range of the host tree. Most of these studies report only on the duration of seed chalcid emergence periods and provide a description of seed cone development at the time of oviposition. Typically, the oviposition period begins soon after emergence (Miller 1916; Hussey 1955; Hedlin 1956) and is thus included in

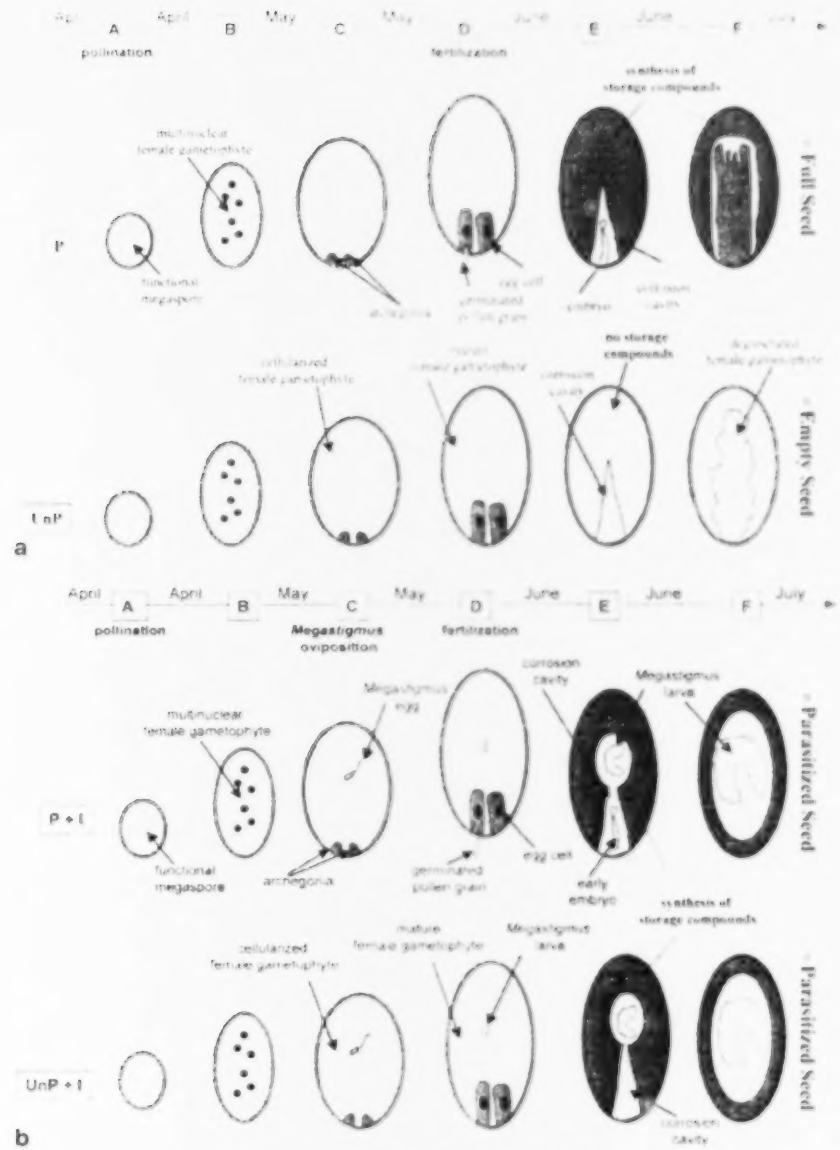

Fig. 4 Schematic development of Douglas-fir ovules: **a** pollinated (*P*) and unpollinated (*UnP*); **b** pollinated infested (*P + I*) and unpollinated infested (*UnP + I*) by *Megastigmus spermotrophus*. Only the female gametophyte is drawn for more clarity. Initiation of the female gametophyte and meiosis occurred earlier in March. The stages shown occur from mid-April to the end of June. Female gametophyte is in *light grey* if no synthesis of storage compounds is observed and in *dark grey* when synthesis begins. *A* functional megaspore (mid-April), pollination period, *B* multinuclear female gametophyte resulting from nuclear division without cell wall formation, *C* cell-wall formation and archegonia differentiation (mid-May); *M. spermotrophus* oviposition period in parasitized treatments, *D* fertilization of the egg cells in P treatment (early June); female gametophyte develops in the same way in P and UnP ovules, egg cells mature in both treatments. In parasitized ovules, egg hatches and larva begins to feed on female gametophyte, *E* in P ovules early embryo grows in the corrosion cavity; it is soon eaten by larvae in P + I ovules; synthesis of storage compounds in female gametophyte cells is observed in both P and P + I ovules. In UnP ovules, a corrosion cavity forms but there is no synthesis of storage compounds by the female gametophyte cells; whereas in UnP + I ovules, a larva grows and the female gametophyte stores lipids, proteins, and starch as in fertilized ovules, *F* in P ovules, the plant embryo develops, consuming female gametophyte (end of June), these seeds are "*filled seeds*" seen in Fig. 3; in UnP ovules, the female gametophyte degenerates and ovule development aborts, giving "*empty seeds*" (Fig. 3); in the presence of a larva, the female gametophyte continue to synthesize storage compounds which the larva feeds on. Soon the larva occupies the whole seed cavity in both parasitized treatments

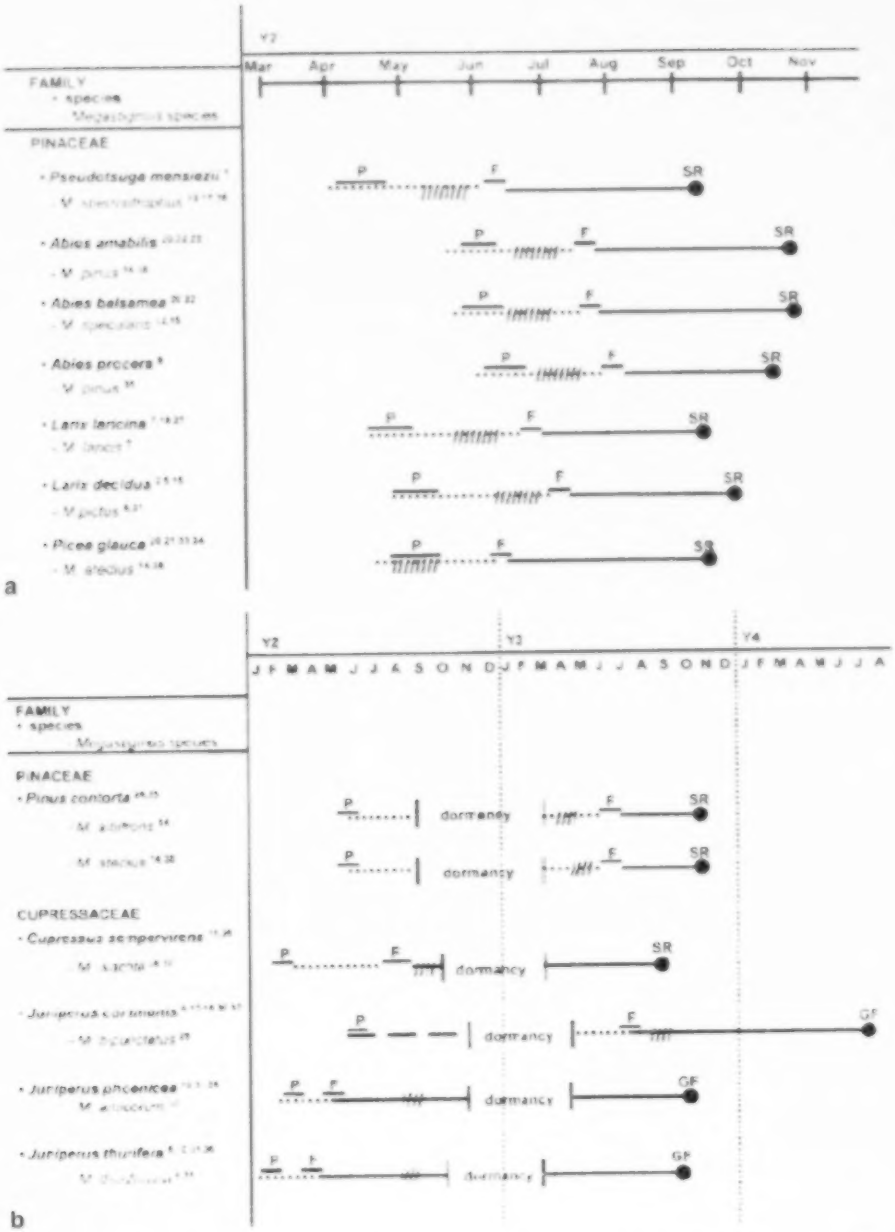

Fig. 5 Relationship between *Megastigmus* oviposition period and ovule development in conifers of the Pinaceae and Cupressaceae: **a** Pinaceae with a 2-year reproductive cycle; **b** Pinaceae with a 3-year reproductive cycle and Cupressaceae with a 3- or 4-year reproductive cycle. Abbreviations and symbols: (*P*) pollination, (*F*) fertilization, (*SR*) seed release, (*GF*) galbule fall, *dashed line* represents time between female gametophyte initiation and beginning of development, *dotted line* represents female gametophyte development, *diagonal lines* (*////*) represent *Megastigmus* oviposition period, *solid line* represents seed maturation, *vertical lines* (|| ||) represent the beginning and end of the period of seed dormancy, *Yn* is number of years of ovule development since floral initiation. *Numbers in superscript text* next to the conifer or *Megastigmus* species names correspond to reference number cited as follows: 1. Allen and Owens 1972, 2. Barner and Christiansen 1960, 3. Blake et al. 1989, 4. Burns and Honkala 1990, 5. Chesnoy 1966, 6. Da Ros 1997, 7. Eavy 1987, 8. El Alaoui El Fels and Roques 2004, 9. Franklin and Ritchie 1970, 10. Gaussen 1968, 11. Giannini et al. 1999, 12. Hedlin 1956, 13. Hussey 1955, 14. Keen 1958, 15. Kettela 1967, 16. Kosinski 1986, 17. Miller 1916, 18. Ottley 1909, 19. Owens 1995, 20. Owens and Blake 1985, 21. Owens and Molder 1979, 22. Owens and Molder 1985, 23. Owens and Morris 1998, 24. Owens et al. 1981, 25. Owens et al. 1982, 26. Pichot 2000, 27. Powell and Tosh 1991, 28. Roques 1981, 29. Roques 1983, 30. Roques and Raimbault 1986, 31. Roques et al. 1984, 32. Rouault 2002, 33. Runions and Owens 1999a, 34. Runions and Owens 1999b, 35. Scurlock et al. 1982, 36. Lemoine-Sébastian 1968, 37. Singh 1978, 38. Speers 1975. Taxonomic authority not given in text: **Conifers** – *Abies amabilis* Dougl. ex Forbes; *A. balsamea* (L.) Mill., *Larix laricina* (Du Roi) K. Koch., *Juniperus communis* L., *J. phoenicea* L., *J. thurifera* L. **Seed chalcids** – *Megastigmus amicorum* Bouček, *M. lupunctatus* (Swederus), *M. laricis* Marcovitch, *M. specularis* Walley, *M. thuriferana* Roques and El Alaoui

the period of emergence. When studies on conifer host and associated seed chalcid were not conducted on the same site, we always chose studies conducted in the same country at low altitudes.

Comparative phenology of chalcid oviposition and ovule development in conifers

Superimposition of the phenologies of seed chalcid oviposition and conifer reproductive development has revealed that the oviposition period of *Megastigmus* species occurs either before or after ovule fertilization (Fig. 5a, b). For *Megastigmus* species exploiting Pinaceae, oviposition always seems to occur before ovule fertilization, during the female gametophyte development, irrespective of the duration of the cone's reproductive cycle (i.e., 2 or 3 years). This pattern suggests that all species of seed chalcid exploiting this family of conifers are likely to behave like *M. spermotrophus*, and thus oviposition in unfertilized seed would be possible. Hussey (1955) suggested that seed fertilization was a cue used by females to select seeds in which to oviposit; but the fact that oviposition begins prior to seed fertilization (Roques 1989) is a clear indication that seed fertilization is unlikely to provide cues usable by females to select oviposition sites.

The *Megastigmus* species exploiting Cupressaceae, on the other hand, reportedly oviposit after the period of ovule fertilization, when the female gametophyte is already fully developed and embryogenesis has begun. Thus, in this family of conifers, seed chalcids would only oviposit in fertilized, rather than in unfertilized, ovules.

Systematic dissections of *P. menziesii* female gametophytes starting at the time of pollination revealed that oviposition by *M. spermotrophus* occurred only after cell wall formation had begun (G. Rouault, unpublished data) and have confirmed that seed chalcid eggs are deposited directly inside the female gametophyte. The female gametophyte of *Picea glauca* (Moench) Voss is already cellular at pollination (Runions and Owens 1999a), when *Megastigmus atedius* Walker oviposits (Fig. 5a). This early onset of cell-wall formation may explain why *M. atedius* is the only species among those exploiting pinaceous trees that apparently oviposit during the pollination period—the remainder oviposit after the pollination period. One noteworthy difference between the oviposition phenology of *Megastigmus* species exploiting Pinaceae with 2- and 3-year fertilization reproductive cycles is that chalcids exploiting those with 3-year cycles begin oviposition following a period of cone and seed dormancy: oviposition occurs in spring, when female gametophyte development resumes. Ovules of *Pinus contorta* Dougl. Ex Loud. undergo winter dormancy at the free nuclear stage (Owens et al. 1981, 1982; Owens and Blake 1985), but cell-wall formation is already underway the following spring at the time of *Megastigmus albifrons* Walker and *M. atedius* oviposition (Fig. 5b). Based on these three systems, we hypothesize that, for all species of Pinaceae, chalcid oviposition occurs only after cell-wall formation has started. At the time when *Megastigmus* species apparently oviposit in Cupressaceae, the female gametophyte already appears fully developed; however, the stage of conifer embryo development is unknown and so is the location where the eggs are deposited.

Oviposition in unpollinated ovules of conifers

The complete development of *M. spermotrophus* larvae in ovules of *Pseudotsuga menziesii* (Niwa and Overhulser 1992; Rappaport et al. 1993; Roux 1995; Rouault 1998) protected from pollination by pollen-exclusion bags, indicates that ovule pollination, as well as ovule fertilization, are not requirements for oviposition and larval development. Circumstantial evidence of larval development in unpollinated ovules of some other Pinaceae with a 2-year cycle has been reported. For example, based on statistical comparisons between the respective numbers of filled, chalcid-infested, and empty seeds (Fig. 2) among *Megastigmus*-infested and sound cones, Da Ros (1997) concluded that some individuals of *Megastigmus pictus* (Förster) were likely to develop in unpollinated ovules of *Larix decidua* Mill. Some *Abies* species are also recognized as poor cone producers, with a high proportion of "empty" seeds (Scurlock 1978; Owens and Molder 1985). Shea (1989) showed that the proportion of *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. seeds infested by *Megastigmus pinus* Parfitt and *Megastigmus rafni* Hoffmeyer were stable among years despite extreme variations in pollination success and abundance of seed cones. On *Abies procera* Rehd., Scurlock et al. (1982) suggested that *M. pinus* can develop fully in unfertilized seeds. The relationship between the oviposition phenology for all of these species of *Megastigmus* and host development is similar to that of *M. spermotrophus* on *Pseudotsuga menziesii*.

For the *Megastigmus* spp. infesting both *Pinus contorta* and *Picea glauca*, oviposition also occurs before fertilization (Fig. 5a, b); however, unlike the other Pinaceae, both host genera reportedly require the presence of pollen for female gametophyte development to proceed (McWilliams 1959; Owens et al. 1981, 1982; Owens and Blake 1985). For both these conifer genera, like for all other Pinaceae, the female gametophyte degenerates if fertilization does not occur. There are no data on development of seed chalcids in unpollinated ovules of *Pinus* and *Picea* species. In both genera, for the eggs to be laid into a developing female gametophyte, one would expect oviposition to occur only in pollinated ovules, assuming that females can differentiate between unpollinated and pollinated ovules. Thus, we hypothesize that in *Pinus* spp. and *Picea* spp., if pollination is required for female gametophyte initiation and then for seed chalcid oviposition, fertilization should not be a requirement for *Megastigmus* to oviposit.

For all the species of *Megastigmus* ovipositing in Pinaceae ovules before fertilization, it is likely that the presence of a *Megastigmus* egg or larva can prevent the

female gametophyte from aborting (Fig. 4b–*UnP+I*). Two mechanisms can be proposed to explain the absence of female gametophyte abortion in unfertilized ovules of conifers parasitized by larvae of *Megastigmus*: (1) a mechanical or chemical action linked to the insertion of the ovipositor into the female gametophyte, or(2) a chemical action from the immature stages within the female gametophyte, as observed in gall insects (Rohfritsch 1992). In Pinaceae, the beginning of female gametophyte development before chalcid oviposition is the major difference between this family and the Cupressaceae, in which oviposition occurs after fertilization in a mature female gametophyte likely containing storage compounds and seed embryo. Larvae of *Megastigmus* associated with Pinaceae begin to develop in an immature female gametophyte before the beginning of the synthesis of storage reserves (Fig. 4b–*UnP+I/D*). To elucidate the possible effect of the larva on the female gametophyte, it is necessary to determine whether chemical relationships between larvae and female gametophyte are different for *Megastigmus* spp. ovipositing before and after fertilization. A biochemical analysis of pollinated and unpollinated, then fertilized and unfertilized, female gametophytes, with and without a larva, would improve our understanding of the influence of *Megastigmus* spp. on female gametophyte physiology and development.

Pollen is required to initiate female gametophyte development of the Cupressaceae (Singh 1978; Owens and Blake 1985; Owens et al. 1990; Arista et al. 2001). In *Cupressus* and in *Juniperus* species, oviposition by *Megastigmus* spp. occurs after fertilization (Roques et al. 1984; Rouault 2002; El Alaoui El Fels and Roques 2004; Fig. 5b). By the time that *Megastigmus wachtli* Seitner is able to oviposit on *Cupressus sempervirens* L., seeds available within a cone either contain a fully developed female gametophyte or are empty, as a result of female gametophyte abortion (Rouault 2002). It is likely that *Megastigmus* spp. cannot oviposit in unfertilized ovules of Cupressaceae. Whether *Megastigmus* spp. ovipositing in Cupressaceae ovules can differentiate between fertilized and unfertilized ovules is unknown.

In conclusion, based on the compiled information, we hypothesized that all species of *Megastigmus* infesting Pinaceae oviposit before fertilization and thus do not require fertilized ovule as oviposition site, because female gametophytes begin to develop in all ovules whether pollinated or not. In *Pinus* and *Picea* species, pollination appears to be necessary for the presence of the female gametophyte, but this requirement is not inconsistent with, nor does it preclude, the possibility that seed chalcids have the ability to oviposit in unfertilized ovules. *Megastigmus* species parasitizing Cupressaceae oviposit only in fertilized seeds already containing a fully developed female gametophyte and a young embryo. To test our hypotheses and predictions, additional studies on seed development in general, and the female gametophyte in particular, similar to those conducted for *M. spermotrophus* on *Pseudotsuga menziesii* are needed for many more *Megastigmus* /conifer systems. These studies must encompass systems from all three basic types of reproductive cycles and from both families of conifers (i.e., Pinaceae and Cupressaceae). One of the implications of our hypotheses is that all seed chalcids attacking Pinaceae, and not only *M. spermotrophus*, can select any of the potential seed within a cone as an oviposition site. This ability suggests a more simple and efficient oviposition strategy than the one where it was believed that females had to locate a fertilized gametophyte. A more general selection is more efficient because it would reduce the amount of time required to find suitable oviposition sites. More oviposition sites would be available, especially in years when pollen production or pollination success is low (i.e., reduce the vulnerability of this insect to variability in time and space of suitable resources in which to oviposit). Another implication is that the current method of calculating chalcid attack rates in Pinaceae leads to an overestimation of their impact on seed production, especially for conifers with high proportions of empty seed. For Douglas-fir, Rappaport et al. (1993) proposed a new formula to calculate attack rates that took into account the ability of *M. spermotrophus* to develop in unfertilized seeds. Whether the same formula could be applied to all other chalcids infesting Pinaceae would have to be investigated.

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